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PROTEIN SYNTHESIS IN NORMAL AND LOBELESS GASTRULAE OF *ILYANASSA OBSOLETA*

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Abstract

Several hundred proteins synthesized by normal and lobeless *Ilyanassa* gastrulae were identified by the two-dimensional electrophoresis of polypeptides labeled *in vivo* with ^{35}S-methionine. Acidic proteins were separated in the first dimension by isoelectric focusing and basic proteins by non-equilibrium pH gradient electrophoresis (NEPHGE).

No qualitative differences were detected among either the acidic or basic polypeptides produced by normal or lobeless gastrulae. These findings show, for those peptides detected in this analysis, that (1) the stage-specific changes in protein synthesis that occur in *Ilyanassa* embryos by gastrulation (Collier and McCarthy, 1981) are not polar lobe dependent, and (2) the polar lobe cytoplasm does not qualitatively affect the expression of either maternal or embryonic mRNAs during gastrulation.

Introduction

Previous observations on protein synthesis during the embryogenesis of the marine mud snail *Ilyanassa obsoleta* have been restricted to early and late cleavage stages (Brandhorst and Newrock, 1981; Collier, 1981a; Collier and McCarthy, 1981) or to later stages of organogenesis (Collier, 1983). Major qualitative and quantitative changes in protein synthesis occur during *Ilyanassa* embryogenesis between the 4- and 25-cell stages of development (Collier and McCarthy, 1981). However, it is not clear whether removal of the polar lobe, a vegetal region of egg cytoplasm essential for normal development, influences gastrular protein synthesis. An answer to this question is important because maternal mRNAs that may have been segregated into different regions of the embryo may not be expressed until a later stage of development, such as gastrulation.

This study clarifies the role of the polar lobe in gastrular protein synthesis. Observations on the basic proteins synthesized by normal and lobeless gastrulae are also included.

Materials and Methods

Maintenance of snails and rearing of embryos

Snails collected from Plumb Beach in Brooklyn, New York were maintained at Brooklyn College in tanks of recirculating sea water. When fed a fresh clam on alternate days the snails laid fresh egg capsules daily. Embryos were reared at 19°C in filtered sea water (obtained from the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachusetts) containing 200 micrograms per ml of streptomycin and penicillin. Polar lobes were isolated from eggs in the trefoil stage by

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agitation in Ca-Mg-low sea water (Collier, 1981b). Normal and lobeless embryos were obtained by pooling eggs from several egg capsules.

Labeling of proteins and sample preparation

Proteins were labeled *in vivo* by incubating 200 to 400 embryos at 19°C for 4 h in 120 microcuries per ml of L ^{35}S-methionine (New England Nuclear, 800–1100 Ci/mmole). After incubation with radioactive precursor the embryos were rinsed in Ca-Mg-free sea water, homogenized with a Dounce homogenizer in 0.02 *M* Tris, pH 8.8, containing 0.002 *M* $CaCl_2$ and 0.05 mg/ml micrococcal nuclease, incubated at room temperature for 3 minutes, made 2% with mercaptoethanol, quick frozen, and lyophilized. The lyophilized homogenate was dissolved in 0.1 ml of sample buffer containing 8.5 *M* urea (Schwarz-Mann Ultrapure), 2.0% NP-40 (Tergito, type NP-40 from Sigma Chemical Co.), 0.1 *M* dithiothreitol, 0.4% pH 3–10 ampholyte, 1.6% pH 5–7 ampholyte (all ampholytes were from Bio-Rad Laboratories), and stored at −70°C.

The radioactivity of all samples was determined following precipitation with trichloroacetic acid (TCA). The acid precipitates of protein were washed with TCA, collected on nitrocellulose membranes (Millipore Co.), and counted in a scintillation spectrometer.

Electrophoresis

Samples of protein frozen in sample buffer were thawed, and electrophoresis in the first dimension (isoelectric focusing) was carried out as described by O'Farrell (1975) with a final ampholyte concentration of 2.8% (1.6% pH 5–7; 0.4% pH 3–10; 0.4% pH 3–5; and 0.4% pH 7–9). Focusing gels were polymerized and run in 20 × 0.15 cm glass tubes and electrophoresis was at 25°C for 19,000 volt hours. These focusing gels had a pH gradient from 5.0 to 7.0.

Basic proteins were separated in the first dimension by nonequilibrium pH gradient electrophoresis (NEPHGE) as described by O'Farrell *et al.* (1977). For NEPHGE electrophoresis sample preparation, composition, and size of focusig gel was as described above except for changes in the arrangement of buffer reservoirs and the polarity of electrophoresis. The cathode buffer chamber containing 0.02 *N* NaOH was placed on the bottom of the electrophoresis unit, and the anode buffer chamber containing 0.01 *N* phosphoric acid was placed at the top of the electrophoresis unit. The sample was loaded at the anode end of the gel and during electrophoresis the basic proteins migrated into the gel and toward the cathode, rather than into the cathode buffer chamber as in isoelectric focusing. Further, electrophoresis was continued for only 1200 VH, *i.e.*, much less than required to reach equilibrium, in order to retain the basic proteins on the gel. The gels used for isoelectric focusing had an effective pH gradient from 7.3 to 8.7.

Second dimension electrophoresis was on 10% polyacrylamide separating gels prepared and run according to Laemmli (1970). Gels were prepared for fluorography as described by Garrells (1979), dried, and exposed to Kodak X-Omat film for the times indicated in the figure legends.

Results

In *Ilyanassa* the gastrula is formed by epiboly of the micromeres over the yolky macromeres. Gastrulation, as determined by the maximal closure of the blastopore, is completed after about 54 hours of development at 19°C (Collier, 1976). When the

polar lobe is removed from *Ilyanassa* eggs the time and mode of gastrulation is essentially the same as in the normal embryo.

Segregation of ectoderm, mesoderm, and endoderm occurs shortly after the formation of the primary mesentoblast cell (the 4d cell), which appears after one day of development at 19°C. The morphogenetic movements that form the gastrula are complete just after the second day of development as described above. Collier and McCarthy, (1981) suggested that the 24-hour embryo, which has approximately 29 cells, be called a mesentoblast embryo and that the older embryo which has completed the morphogenetic movements required for the formation of the blastopore be called a gastrula.

Figures 1A, B are autofluorographs of radioactive polypeptides produced, respectively, by normal and lobeless *Ilyanassa* gastrulae. These radioactive peptides were separated by two-dimensional electrophoresis on polyacrylamide gels. They were extracted separately from control and lobeless embryos that were reared to gastrulae and incubated in ^{35}S-methionine. Some apparent discrepancies among the radioactive spots in these fluorographs are indicated by arrows in Figure 1A. For example, spots that appear to be present in Figure 1A but absent in Figure 1B are indicated by long arrows, and one spot smeared in Figure 1B but clearly resolved in Figure 1A is marked by a short arrow. These discrepancies are artifacts because they were not observed consistently among gels prepared from different protein preparations nor among replicate electrophoretic separations from the same protein sample.

About 685 individual radioactive spots are resolved on each of the autofluorographs in Figures 1A, B. These spots correspond to polypeptides that were translated from the more abundant classes of mRNAs of normal and lobeless *Ilyanassa* gastrulae. That this is only a partial detection of translatable mRNAs is evident from considerations of the sensitivity of resolution and detection of radioactive peptides by two-dimensional electrophoresis, the distribution of methionine among proteins, and the limits of the pH gradient, *i.e.*, pH 5 to 7, of the isoelectric focusing gels. For example, in regard to the latter point it has been estimated by Tufaro and Brandhorst (1979) that more than 85% of ^{35}S-methionine-labeled sea urchin proteins enter isoelectric focusing gels in the pH range of 4.5 to 7.2. Thus, the observations (Figs. 1A, B) on proteins separated from several groups of normal and lobeless *Ilyanassa* gastrulae demonstrate the similarity of proteins produced by these two classes of embryos at gastrulation.

Figures 2A, B are autofluorographs of *in vivo* labeled proteins extracted from normal and lobeless gastrulae, respectively, and resolved by NEPHGE. This method of electrophoresis resolves basic proteins that either do not enter or fail to be resolved in isoelectric focusing gels with acidic and neutral pH gradients. Because NEPHGE gels are not focused to equilibrium, only proteins in the basic regions (pH 7.3–8.7) of the gel are resolved. Although this pH gradient extends more into the alkaline pH range than the gradient used in equilibrium isoelectric focusing, it is not satisfactory for the separation of histones, which would, in the presence of urea, require a gradient from pH 8.9 to 10.25 (Valkonen and Piha, 1980). Gels focused to equilibrium show some apparent differences among the radioactive spots displayed in Figure 2. However, these discrepancies were not seen consistently among replicate gels. Therefore, this analysis shows the similarities of the basic polypeptides produced by normal and lobeless embryos.

Discussion

If maternal mRNAs are differentially expressed, either by differential localization, translation, or destruction, in different blastomeres or regions of the embryo (Tufaro

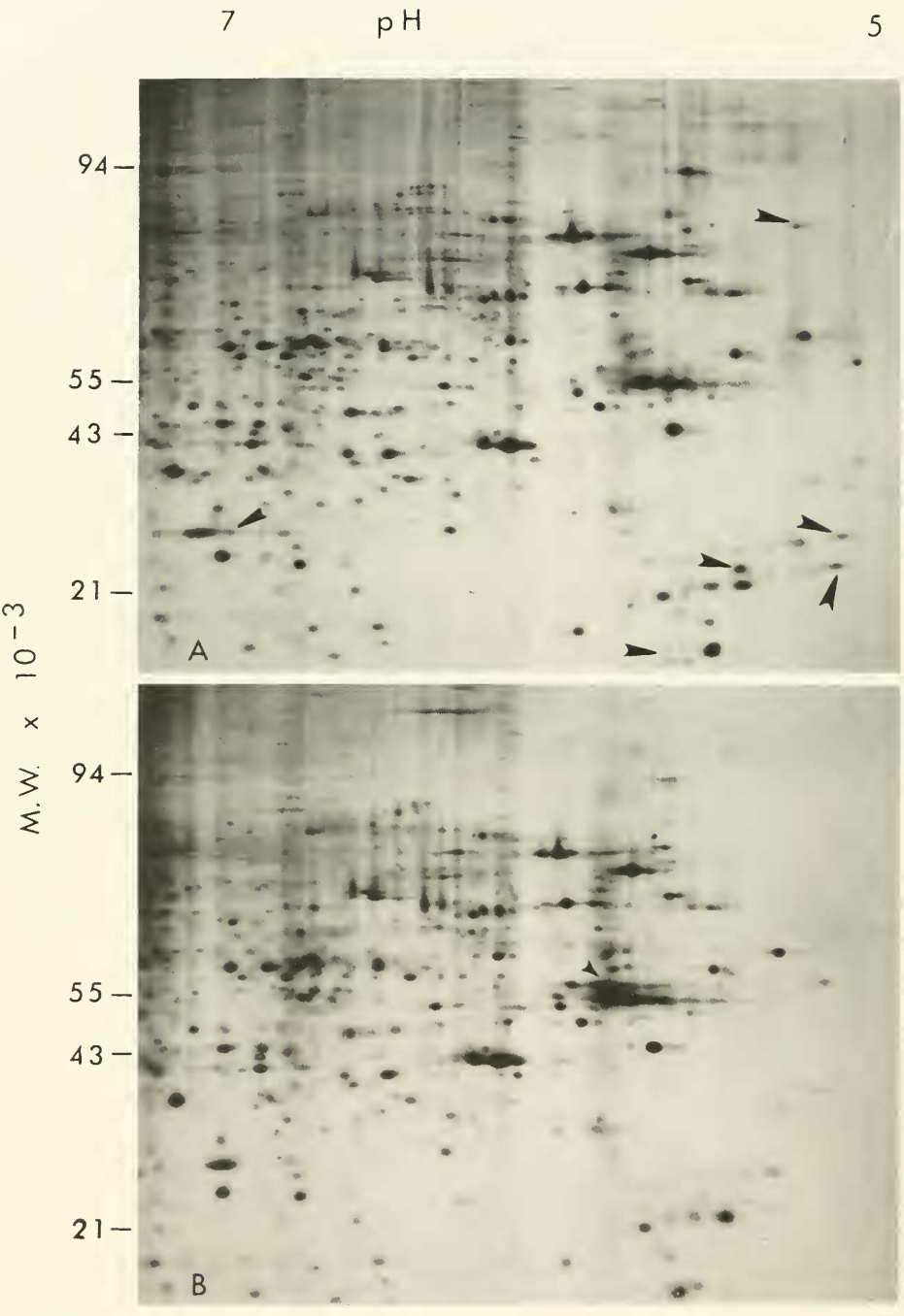

FIGURE 1. Autoradiographs of ^{35}S-methionine labeled acidic proteins synthesized by (A) normal, and (B) lobeless gastrulae. The large arrows in 1A indicate spots that were not seen consistently among replicate gels. The small arrow in 1B marks a spot smeared in this figure but clearly resolved in 1A.

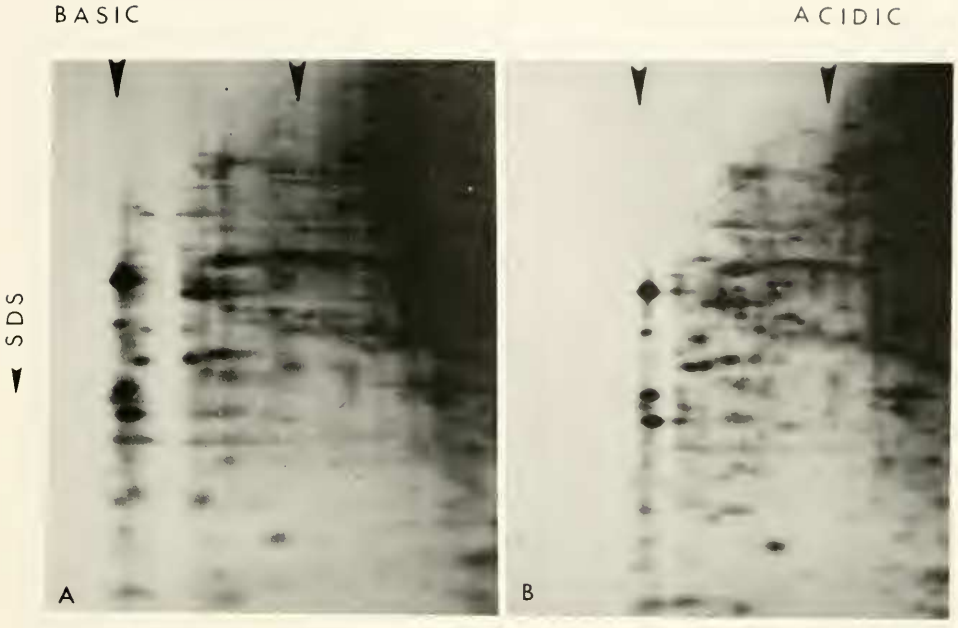

FIGURE 2. Autoradiographs of ^{35}S-methionine labeled basic proteins synthesized by (A) normal, and (B) lobeless gastrulae. Vertical arrows mark the basic region (pH 7.3–8.7) of the NEPHGE autoradiograph in which polypeptides were resolved.

and Brandhorst, 1979; Brandhorst *et al.,* 1983; Collier, 1983; Jeffery, 1983), one may expect that this expression could occur at any of several stages of development. Analyses of protein synthesis during early stages of *Ilyanassa* development have failed to detect a differential expression of mRNAs. Therefore it became important to ask if the expression of some mRNAs may have been delayed until a later period in development, *e.g.,* gastrulation.

The results reported here demonstrated no detectable qualitative differences among 685 polypeptides produced by normal and lobeless gastrulae. A number of basic non-histone proteins were also found to be similarly produced by both normal and lobeless gastrulae. All of these proteins were translated from the prevalent and moderately prevalent classes of gastrular mRNAs. They do not include proteins coded for by the rare or complex classes of mRNA which are not detected by two-dimensional electrophoresis of proteins labeled *in vivo* with ^{35}S-methionine. Therefore, it is clear that the polar lobe cytoplasm does not have a delayed effect on the expression of maternal mRNAs. Similarly, the polar lobe does not qualitatively influence the expression of embryonic mRNAs that are produced and translated during gastrulation.

From these observations it may also be deduced that the stage-specific changes in protein synthesis that occur in the *Ilyanassa* embryo prior to gastrulation (Collier and McCarthy, 1981) are not dependent on the polar lobe cytoplasm because these changes occur in both normal and lobeless gastrulae.

These and earlier studies of protein synthesis in *Ilyanassa* embryos, have shown no obvious correlation between qualitative changes in protein synthesis and the developmental events influenced by the egg cytoplasm in the polar lobe. Earlier studies (Donohoo and Kafatos, 1973; Newrock and Raff, 1975) on protein synthesis in *Ilyanassa,* which suggested a differential expression of maternal mRNAs, have not

been confirmed by the recent work of Brandhorst and Newrock (1981) and Collier and McCarthy (1981). The observations of Cheney and Ruderman (1978) on *Spisula* embryos have not been published in sufficient detail for appraisal. No qualitative differences in the pattern of protein synthesis have been detected during determinative stages of sea urchin embryogenesis, *e.g.*, the micro-, meso-, and macromeres of the 16-cell (Tufaro and Brandhorst, 1979) and vegetalized sea urchin embryos (Hutchins and Brandhorst, 1979). Therefore, the available data suggest that the developmental events that implement determination are not reflected by qualitative changes in the synthesis of the abundant classes of proteins which are readily detectable by two-dimensional electrophoresis of *in vivo* labeled proteins. However, the finding of posterior indicator and head foretelling proteins in *Smittia* embryos by Jackle and Kalthoff (1980, 1981) are in striking contrast to the absence of qualitative changes in protein synthesis during the determinative stages of *Ilyanassa* and sea urchin embryos.

In a previous discussion (Collier, 1983) of the relation of quantitative differences in protein synthesis to differentiation, it was pointed out that the greatest quantitative disparity in protein synthesis between normal and lobeless embryos was among the most abundant proteins, *i.e.*, those most rapidly labeled. This observation, as well as those of Harkey and Whiteley (1982) on sea urchin embryos, shows that the major quantitative differences that occur during differentiation and organogenesis are developmentally regulated. In view of the failure to establish a correlation between qualitative changes of this class of proteins and differentiation, it is reasonable to think that these proteins are ubiquitously required by all cells and that their role in differentiation is achieved by quantitative variations among different cell types.

If the determinative events of early embryogenesis involve the differential localization of maternal or embryonic mRNAs and the prevalent classes of mRNA are excluded, then one must search for these determinants among the rare and complex class of mRNAs. The rarity and complexity of these mRNAs, as estimated for sea urchin embryos (Galau *et al.*, 1976; Davidson and Britten, 1979; and Duncan and Humphreys, 1981), are, respectively, 1–15 copies per cell and 12,000 to 14,000 individual species of mRNAs. To approach this problem requires subfractionation of proteins prior to electrophoresis in order to resolve 12,000 to 14,000 radioactive spots on 150 to 200 square centimeters of polyacrylamide and the removal of the abundant class of proteins, which obscures the registry of the rare species of labeled proteins on an autoradiograph. Finally, the distribution of oogenetic proteins among the early blastomeres should be considered in a search for molecules related to determination.

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